Selectivity issues in affinity-based biochemical sensors: Determining the ratio of similar biomolecules in binary mixtures

Ivana Jokić, Olga Jakšić, Zoran Jakšić, *Senior Member, IEEE*,
Katarina Radulović and Miloš Frantlović

***Abstract*— We consider selectivity of affinity-based nanosensors utilizing resonance shift due to the presence of adsorbed analyte. Among such devices we analyze mass-based sensors utilizing mechanical resonance in e.g. micro or nanocantilevers and all-optical refractometric sensors utilizing surface plasmon polariton resonance. The sensitivity of such devices can be extremely high, reaching single-molecule level, however their selectivity is limited by the differences in mass in the first case or in refractive index values between different analytes in the second. The typical approach is to use some kind of receptors on the sensor surface with highly specific binding of a targeted analyte. The properties of a given biomolecule, for instance protein, will vary between its different conformations due to different arrangements of their atoms in space. Since the conformation of a molecule is critically important for its function, it is of interest to determine the ratio between different conformational isomers in a given sample. In general, different conformations of a biomolecule may have different affinity toward binding sites on the surface of an affinity-based nanosensor, as well as different surface-volume ratios. We argue that the analysis of adsorption kinetics ensures sufficient data to discriminate between different conformations in a mixture.**

***Index Terms*—Biochemical sensors; Affinity Sensors; Microcantilevers; Surface Plasmon Polaritons; Refractometric Devices; Adsorption Kinetics; Desorption; Sensor Selectivity.**

I. Introduction

DETECTION of chemical and biological (CB) analytes is among the important goals of modern sensor technologies [1, 2]. The need to recognize the presence and quantify the amount of a given species within a mixture of an arbitrary complexity has a large importance in different fields. These include the detection of chemical and biological agents in homeland security and anti-terrorist actions, where it is necessary to track down trace amounts of toxic and explosive chemicals preferably in real time. Another field is environmental protection, with the need to determine the presence of dangerous pollutants, especially those remaining in the food chain. Yet another important area is the detection of different species in process industry, an example being the detection of aromatic and other hydrocarbons in oil and gas industry, etc.

It may be generally said that the detection of an analyte is based on its affinity for binding to a certain recognizing element, which in turn causes the change of a sensor measuring parameter. The nature of this parameter may be e.g. mechanical, optical or electrical. Typically an analyte will bind to adsorption sites on the surface or within the volume of a sensing element. This ensures pre-concentration of the analyte on the surface or within the volume of the sensor and increases the sensitivity compared to the device where simple immersion to mixture containing analyte is used. At the same time, the affinity process, if properly targeted, can increase the selectivity of the process.

Among different CB sensors probably the most sensitive ones are those based on some kind of resonance. The presence of analyte modifies the conditions for the resonance and thus shifts the sensor resonant peak. In this manner even the minute changes of the analyte concentration may cause large changes of output signal.

The main kinds of resonance-based CB sensors are those utilizing mechanical resonance or electromagnetic/optical resonance. The example of the first kind of sensors are MEMS or NEMS produced mechanical oscillators [3], typical examples being micro and nanocantilever, micro and nanobridges and nanomembranes. The adsorption of analyte increases the overall mass of the oscillating mechanical element, while desorption decreases it, which is felt in the shift of the resonant frequency of the oscillator. A typical example of the second kind of sensors are devices utilizing surface plasmon polaritons (SPP), where resonance conditions are met between free charge carriers within a conductor (plasmon) and polarized electromagnetic wave propagating at the interface between the conductor and the ambient containing analyte (polariton) [4]. The adsorbed analyte modifies the propagation of the evanescent surface

Ivana Jokić is with Centre of Microelectronic Technologies, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia (e-mail: ijokic@nanosys.ihtm.bg.ac.rs).

Olga Jakšić is with Centre of Microelectronic Technologies, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia (e-mail: olga@nanosys.ihtm.bg.ac.rs).

Zoran Jakšić is with Centre of Microelectronic Technologies, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia (e-mail: jaksa@nanosys.ihtm.bg.ac.rs).

Katarina Radulović is with Centre of Microelectronic Technologies, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia
(e-mail: kacar@nanosys.ihtm.bg.ac.rs).

Miloš Frantlović is with Centre of Microelectronic Technologies, Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia (e-mail: frant@nanosys.ihtm.bg.ac.rs).

electromagnetic wave by changing the value of the refractive index at the surface. This type of CB sensors includes nanoplasmonic devices [5], where metal-dielectric is structured on nanometer (i.e. subwavelength) level. This type of sensors can be further generalized to electromagnetic metamaterials, where the wavelength range used may be extended from UV-visible to infrared and terahertz ranges and even further [6-8].

In all resonant CB sensors the measured output is proportional to the number of adsorbed analyte molecules. Sensor selectivity for a target analyte is usually achieved through the surface functionalization by some kind of receptors ensuring highly specific adsorption of the target molecules. All resonant CB sensors are fabricated utilizing micro or nanofabrication. Their joint property is a very high sensitivity, which is a natural consequence of their resonant operation. It has been reported that experimental nanoplasmonic sensors based on plasmonic nanoantennas have reached the single molecule sensitivity [9]. Real time measurement of the nanocantilever resonance frequency shift caused by adsorption of a single protein molecule is also demonstrated [10].

One of the situations that occur in chemical/biochemical sensing is the existence of conformational isomers of analytes. A detected molecule may have the same analytical formula, but different spatial (3D) molecular orientations, i.e. different arrangements of atoms in space. Conformational isomers are one subgroup within this wider group where one can convert one isomer into another solely by using rotation about single bonds. Among the typical examples are proteins, where different conformations occur caused by ambient changes, e.g. temperature, pH factor, chemical composition of the solution, etc. The ratio of isomers is critically important for their function in biological systems. Different conformations perform different functions. For instance, in the case of amino-acid alanine, its L-isomer is widely present and is critical for life function, while D-isomer is rarely found, mostly in bacteria and in some antibiotics. It is thus crucial to know which protein conformation dominates in the analyzed sample, i.e. what is the percentage of each conformation in the mixture.

Different conformations of a protein mean different surface-to-volume ratio, as well as different adsorption parameters, i.e. different affinities for the same binding sites/functionalizations. This can be utilized for characterization of mixtures of conformational isomers using resonance-based CB sensors [11].

In this paper we investigate the detection of different protein conformations and compare it to the case of homogeneous analyte. We base our methodology on the transient analysis of adsorption of conformational isomers on the sensor surface. The analysis of the influence of the presence of multiple conformations in the sample on the sensor response is illustrated on the example of plasmonic/nanoplasmonic sensors, which enables us to propose a method for the determination of the fraction of each protein conformation in the two-conformation mixture.

II. Theory

Let us assume that a target protein in the analyzed sample appears in two different conformations, which have different affinities for binding sites on the functionalized sensor surface (Fig. 1).

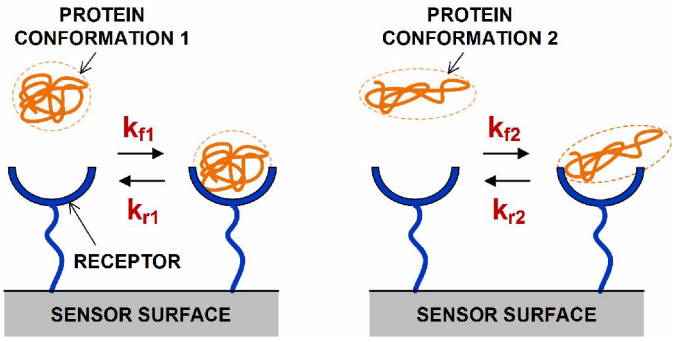

Fig. 1. Schematic presentation of reversible binding of two protein conformations and receptors (molecules used for sensor surface functionalization).

We observe the case of 1:1 binding of a molecule of any of the two protein conformations to the adsorption site. This process occurs under stable experimental conditions, which means that there are no changes of temperature of other parameters of the sample solution that could cause a change of the ratio of isomer concentrations throughout the measurement. If the maximum number of molecules adsorbable at the whole functionalized area is equal for both species (equal to the total number of binding sites N_m) the numbers of bound molecules of the two conformations, $N_1(t)$ and $N_2(t)$ change according to the equations

$$\frac{dN_1}{dt} = k_{f1}C_1(N_m - N_1 - N_2) - k_{r1}N_1 \quad (1)$$

$$\frac{dN_2}{dt} = k_{f2}C_2(N_m - N_1 - N_2) - k_{r2}N_2 \quad (2)$$

Here k_{f1} and k_{f2} are adsorption rate constants of the first and the second protein conformation, respectively, and k_{r1} and k_{r2} are their desorption rate constants. If the total protein concentration in the sample is C, and p is the fraction of the conformation 1 in the mixture, the concentrations of the two protein conformations are $C_1=pC$ and $C_2=(1-p)C$, so that Eqs. (1) and (2) can be written in the form

$$\frac{dN_1}{dt} = k_{f1}pC(N_m - N_1 - N_2) - k_{r1}N_1 \quad (3)$$

$$\frac{dN_2}{dt} = k_{f2}(1-p)C(N_m - N_1 - N_2) - k_{r2}N_2 \quad (4)$$

Their solutions are

$$N_1(t) = N_{10}\left(1 + \frac{(1/k_{r2} - \tau_1)}{(\tau_1 - \tau_2)}e^{-t/\tau_1} + \frac{(1/k_{r2} - \tau_2)}{(\tau_2 - \tau_1)}e^{-t/\tau_2}\right) \quad (5)$$

$$N_2(t) = N_{20}\left(1 + \frac{(1/k_{r1} - \tau_1)}{(\tau_1 - \tau_2)}e^{-t/\tau_1} + \frac{(1/k_{r1} - \tau_2)}{(\tau_2 - \tau_1)}e^{-t/\tau_2}\right) \quad (6)$$

The stationary value of the adsorbed particles number for

the protein with conformation 1 is $N_{10} = k_{f1} pCN_m k_{r2} \tau_1 \tau_2$ and for the protein with conformation 2 it is $N_{20} = k_{f2}(1-p)CN_m k_{r1} \tau_1 \tau_2$. The time constants τ_1 and τ_2 determining the sensor response dynamics are given by

$$\tau_{1,2} = 2/(b \mp \sqrt{b^2 - 4c}) \tag{7}$$

$$b = k_{f1} pC + k_{r1} + k_{f2}(1-p)C + k_{r2}$$

$$c = k_{f1} pCk_{r2} + k_{f2}(1-p)Ck_{r1} + k_{r1}k_{r2}$$

The plasmonic sensor response is determined by the total refractive index change caused by adsorption of both forms of the protein, according to the formula (effective medium approach, simple mixing rule)

$$\Delta n_{eff} = \frac{n_1 - n_e}{N_m} N_1 + \frac{n_2 - n_e}{N_m} N_2 \tag{8}$$

where n_1 and n_2 are refractive indexes of the first and of the second conformation, respectively, while n_e is the refractive index of the surrounding medium.

III. Results and Discussion

In order to investigate quantitatively the effects of adsorption of two conformations of a protein on the plasmonic sensor response, the parameter values are chosen so to correspond to realistic experimental situation: total protein concentration in the sample solution C=1 nM, the number of adsorption sites N_m=3.01·10^6 on a functionalized surface area A=10^{-9} m^2, and the adsorption and desorption rate constants k_{f1}=8·10^7 1/(M·s), k_{r1}=0.08 1/s, k_{f2}=8·10^6 1/(M·s), k_{r2}=0.02 1/s.

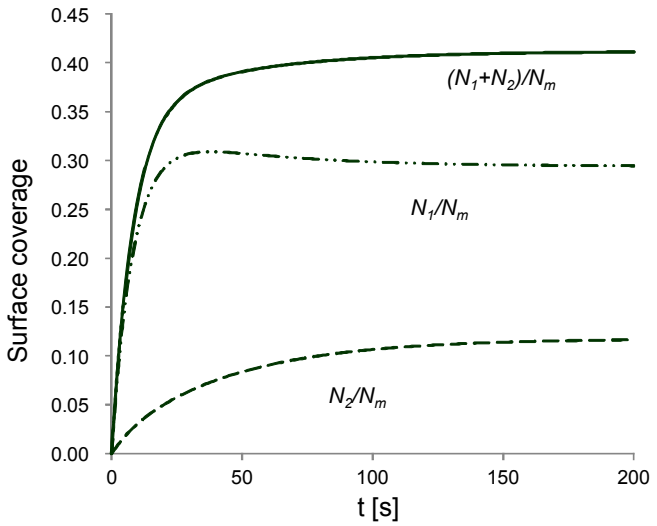

Fig. 2. The time change of the coverage of the functionalized sensor surface by protein molecules of each of two conformations separately and of the total surface coverage.

Fig. 2 shows the temporal evolution of the coverage of the sensing surface by molecules of both conformation 1 (dash-dotted line) and conformation 2 (dashed line), i.e. N_1/N_m and N_2/N_m, respectively, for the case when both protein conformation are present in equal amounts in the sample (p=0.5). The total surface coverage, $(N_1+N_2)/N_m$, is also shown in the diagram (solid line). The adsorption of the conformation 1 is dominant in the beginning and determines the total amount of the adsorbed particles, but the equilibrium state is reached after a certain number of the adsorbed molecules is replaced by the molecules with the conformation 2, whose number varies slowly, but steadily. The adsorption of the conformation 2, which has a lower affinity towards the applied functionalization, determines the time necessary to reach the total equilibrium coverage of the adsorbant surface.

The analysis of the surface coverage gives insight into the adsorption kinetics for the case of two-conformation mixture. However, the response of the plasmonic sensor is determined not only by the number of the adsorbed particles (i.e. by the coverage of the surface by each of the two kinds of adsorbate), but also by the contribution of each of the adsorbed species to the refractive index change. Fig. 3 shows the calculated transient response of plasmonic sensor for nine different compositions of two-conformation mixture ($0<p<1$) and two limiting cases that correspond to a homogeneous sample containing either the analyte 1 (p=1) or the analyte 2 (p=0). The values of refractive index of the two protein conformations are n_1=1.59, n_2=1.61 and n_e=1. A significant variation of the sensor temporal response with variation of the parameter p between the two limiting values is observed (top and bottom curve in Fig. 3). Both the steady-state value and the response time needed to reach the steady state are situated between them. In the case under consideration the equilibrium value of response increases monotonically, while the response time decreases monotonically with p.

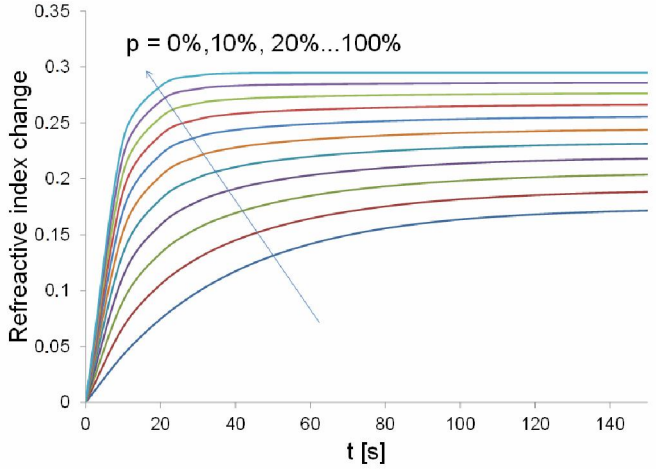

Fig. 3. The plasmonic sensor temporal response for different compositions of two-conformation mixture.

The dependence of the sensor response in equilibrium (reached when all transients are ended) on the fraction of a single conformation in a mixture is shown in Fig. 4. Fig. 5 shows the sensor response time (defined here as the time necessary to reach 99% of the equilibrium value) versus p.

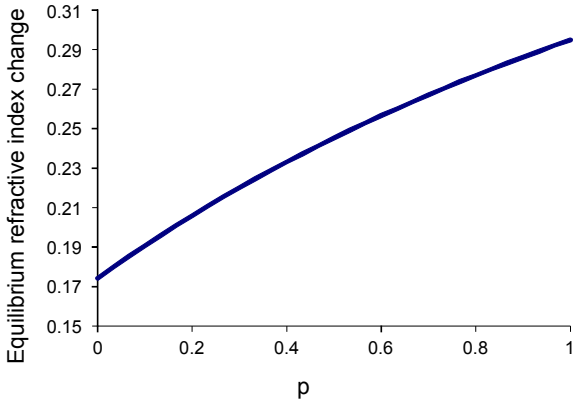

Fig. 4. The dependence of the plasmonic sensor response in equilibrium on the fraction of protein conformation 1 in a mixture.

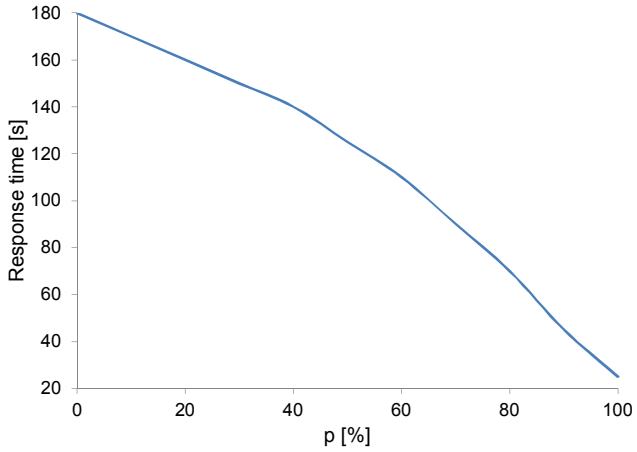

Fig. 5. The sensor response time as a function of fraction of one of two protein conformations in a sample.

It can be seen that both the equilibrium refractive index change and the response time clearly indicate the value of p, i.e. they contain the information on the two-conformation mixture composition. These two parameters of the sensors temporal response also depend on the protein concentration in the analyzed sample. Therefore, based on the derived theory (Eqs. (5)-(8)) and the performed analysis, a method is available for simultaneous determination of the total concentration of the target protein and of the percentage of each of its two conformations in the sample.

IV. Conclusion

One of the realistic situations in biochemical detection is investigated, when it is necessary to quantify the analyte that exists in the sample in various forms, known as conformational isomers. The possibility to extract information from the sensor temporal response in the case of two-conformation mixtures is considered. It is shown that the analysis of sensors response kinetics ensures sufficient data to determine the total analyte concentration and also concentrations of each of the conformational isomers comprising the analyte. The results are obtained for nanoplasmonic sensors where refractive index is modified by the presence of analyte, however they are useful for all adsorption-based sensors, especially those based on resonance. Actually, the results are applicable even wider: adsorption-desorption phenomena influence practically all MEMS/NEMS devices since they must be surrounded by some kind of medium and thus subject to different atomic/molecular species impinging to the surface, while their environment always consists of multiple components. This influence rises with decreasing dimensions, i.e. it is mostly felt in NEMS structures.

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